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(54) Title: THERAPEUTIC COMPOSITION FROM SUGAR CANE AND THERAPEUTIC USES THEREOF

(57) Abstract

The present invention provides therapeutic compositions derived from sugar cane which are useful for treating a variety of pathological conditions in animals, particularly humans, including those caused by inflammation; bacterial, viral, or fungal infection; toxins; trauma such as burns; and other causes. Also provided are methods for preparing the therapeutic compositions, pharmaceutical formulations comprising the compositions, methods for treating pathological conditions, and methods for purifying the active components from the compositions.

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THERAPEUTIC COMPOSITION FROM SUGAR CANE AND THERAPEUTIC USES THEREOF

Field of the Invention

This invention relates to compositions derived from extracts of sugar cane, which are useful to treat a variety of pathological conditions in humans and other mammals.

Background of the Invention

The present invention is based on the unexpected discovery that saccharide-containing extracts of sugar cane prepared according to the methods of the invention are effective for the treatment of a wide variety of pathological conditions. Heretofore, sugar cane and extracts thereof have not been recognized as containing therapeutically beneficial components.

Many pathological conditions involve complex and ill-defined adhesive interactions between oligosaccharide or other carbohydrate moieties present on the surface of biological entities such as extracellular matrix, cells, or viruses. In some cases, lectins or lectin-like molecules are involved; these molecules (usually proteins) specifically recognize, and bind with high affinity to, particular sugar or oligosaccharide moieties, thereby implementing a particular binding or recognition function. Less specific adhesive interactions between surface components may also involve sugar or oligosaccharide moieties. Adhesive interactions may involve monosaccharides, oligosaccharides having highly stereospecific structures, or polysaccharides containing different substitutent side-chain sugars. The interactions are primarily electrostatic, and may be between different sugar moieties, sugars and protein components, or combinations thereof. It has been suggested that drugs that interfere with these interactions may be useful as anti-

inflammatory and anti-infectious agents (Sharon et al., *Scientific American*, Jan., 1993, p. 82).

Without wishing to be bound by theory, it is believed that the treatment methods and compositions of the present invention exert their effect primarily via an anti-adhesive mechanism. That is, it is believed that a beneficial effect is achieved by interfering with specific interactions between cell-surface lectin-type molecules that may be present on one entity, and the corresponding sugar or oligosaccharide moieties recognized by such lectins that may be present on another entity. One entity may be a pathogenic microorganism such as a virus or bacterium, and the other entity may be a cell, such as, for example, a skin or mucosal cell. Alternatively, one entity may be a cancer cell, virus infected cell, or a pathogenic microorganism and the other entity may be normal tissue or a component of the extracellular matrix or environment. In addition, the hygroscopicity of the composition of the present invention is believed to contribute to its beneficial therapeutic and preventive properties.

Prior to the present invention, there would have been no reason to believe that extracts of sugar cane, particularly those prepared according to the invention, would exhibit beneficial anti-adhesive properties.

Summary of the Invention

The present invention provides therapeutic compositions, preferably derived from sugar cane, which contain primarily saccharide and amino acid components and are useful for treatment of a variety of pathological conditions in mammals, including humans. The precise nature of the active principle(s) is not yet known; nonetheless, it is believed that heat polymerization of one or more components of the sugar cane extract is required to develop the therapeutic activity of the composition.

The pathological conditions that may be treated according to the invention encompass those caused by inflammation, by bacterial, viral (including retroviral), or fungal infection, or by toxins, including without limitation dermic lesions produced by Hansen's disease, diabetic foot ulcers, diabetic gangrene, perineal necrotizing cellulitis, intestinal fistula, rectum-perineal fistula, eviscerations, decubital and vascular source ulcers, acne, and infection with herpes simplex, herpes genitalis, influenza and cold viruses, Hepatitis A and B, and human immunodeficiency virus (HIV).

In another aspect, the invention provides methods for preparing compositions useful in anti-adhesive therapies, comprising the steps of:

(i) macerating sugar cane to produce a liquid extract, and filtering the extract;

(ii) heating the filtered liquid extract obtained in (i) at a temperature of from about 60°C to about 70°C for a time period of about 30 to about 60 minutes;

(iii) heating the filtered liquid extract obtained in (ii) at a temperature of from about 130°C to about 165°C for about 24 hours, under agitation; and

(iv) cooling the extract obtained in (iii) to obtain a solid.

In yet another aspect, the invention provides methods for treatment of pathological conditions in humans, which comprise administering effective amounts for treating the conditions of the anti-adhesive compositions.

Administration of the anti-adhesive compositions of the present invention may be achieved by any effective route, including topical, oral, enteral, intravenous, intramuscular, subcutaneous, transmucosal, and by-inhalation routes. The preferred mode of administration depends upon the condition being treated. For example, for treating skin lesions or wounds, a topical route is preferred; while treatment of a systemic viral infection is preferably achieved using oral or intravenous administration.

Also provided are pharmaceutical formulations comprising the compositions described above in conjunction with pharmaceutically acceptable carriers and/or excipients.

In yet another aspect, the invention provides methods for purifying one or more active components from the anti-adhesive compositions and methods for testing the efficacy of purified components (and combinations thereof) in treating different pathological conditions.

Detailed Description of the Invention

All patents, patent applications, and references cited herein are hereby incorporated by reference in their entirety. In the case of inconsistency, the present description, including definitions, will control.

The present invention provides compositions and methods for treating a broad range of pathological conditions in animals, preferably mammals, and most preferably, humans.

The compositions are preferably derived from sugar cane by extraction and heating of the extract in a non-fermentation process, which is described in more detail below. The methods involve administering to affected individuals or applying to the affected organ or tissue effective amounts of the therapeutic compositions of the invention for a sufficient time to achieve a beneficial clinical result.

An "effective amount" of the compound for treating a pathological condition according to the present invention is an amount that results in measurable amelioration of at least one symptom or parameter of the condition. An effective amount for treating the condition can be determined by experimentation known in the art, such as by establishing a matrix of dosages and frequencies and comparing a group of experimental units or subjects to each point in the matrix. The exact amount to be administered to a patient may vary depending on the nature of the disorder, the severity of the disorder, and the physical condition of the patient. A measurable or significant amelioration of any symptom or parameter may be determined by assay as known in the particular field, or determined by a physician skilled in the art, or reported by the patient to the physician. It will be understood that any clinically or subclinically significant attenuation of any symptom or parameter pursuant to treatment according to the present invention is within the scope of the invention. Clinically significant attenuation means perceptible to the patient and/or to the physician.

A "pathological condition" as used herein refers to any dysfunction in a normal physiological function or disruption in general health of the individual. The pathological conditions that may be treated using the compositions of the present invention encompass those caused by inflammation, by bacterial, viral, or fungal infection, or by toxins, as well as other pathological conditions such as burns and other wounds. They include without limitation dermic lesions produced by Hansen's disease, diabetic foot ulcers, diabetic gangrene, perineal necrotizing cellulitis, intestinal fistula, rectum-perineal fistula, eviscerations, decubital and vascular source ulcers, acne, and infection with herpes simplex, herpes genitalis, influenza and cold viruses, Hepatitis A and B, and human immunodeficiency virus (HIV) or its complications such as from opportunistic infections,

pneumocystis carinii, tuberculosis, toxoplasmosis, mycosis fungoides, cytomegalovirus, etc.

Preparation of Active Extract

The compositions of the present invention may be prepared by the following procedure:

1. Sugar cane that has achieved mature growth is harvested.

2. Extraction: The cane, including an intact bark and medulla, is introduced into a sugar mill (trapiche). The cane is thoroughly macerated to produce a liquid extract and a fibrous residue, called bagasse. The liquid is separated from the bagasse and by mechanical means and filtered, and the bagasse is discarded.

3. Heating step #1: The liquid extract is transferred to a heating chamber, such as an oven, in which it is maintained quiescent at a temperature of between about 60-70°C for 30-60 min, preferably 45 min. During this step remaining impurities rise to the top and are skimmed off. The temperature range and heating time are chosen to allow the impurities to rise to the surface.

4. Heating step #2: The heated liquid extract is then heated at temperatures between about 130-165°C, preferably 140-160°C and most preferably 150°C, for a 24-hour period. During this time, the extract is subjected to constant and uniform agitation, which is e.g. achieved using mechanical but gentle means such as a spoon which may be made of wood, metal or another heat resistant material, stirring at a mixing rate of 20-25/RPM; preferably, this is done in an open container most preferably one made of heat-cured laurel wood. In the process, a considerable fraction of the water component of the extract evaporates. The evaporation process is monitored visually and proceeds until the extract has a semisolid or solid consistency and color similar to that of honey. The color of the product is light brown. It is important that the extract reach a temperature of at least 150°C (its boiling point at sea level).

5. Solidification: The thick, heated extract from step 4 is then transferred dropwise to a stirring container, where it is cooled and thereby further thickened. The thick mass is then set preferably in wooden molds until it solidifies.

It is preferred to avoid use of chemicals to process or discolor the extract as the activity is likely to be adversely affected. It is also preferable to recover the extract from freshly harvested sugar cane and processed immediately to avoid fermentation.

The resulting solid, designated Bercedin, has the following chemical composition:

component	weight % (grams)
carbohydrate (mono-, oligo- and polysaccharides)	90
lectin and other amino acid containing molecules	0.6
calcium	0.3
iron	0.51
phosphorus	0.57
thiamines	0.02
niacin	0.42
carotenes	0.17
riboflavin	0.17
ash	0.10
nonash impurities	0.20
fiber	0.20
water	6.74

The present invention encompasses pharmaceutical formulations comprising Bercedin and bioactive substances, and mixtures of substances, derived therefrom (see below). The formulations include liquid dosage forms having a physiologically acceptable carrier, such as, for example, phosphate buffered saline or deionized water. Bercedin is highly soluble in water and can be easily dissolved at concentrations of 10-100% (w/v). In its solid form it is very hygroscopic. The pharmaceutical formulations may also contain excipients, including preservatives and stabilizers, that are well-known in the art. Bercedin or its derivatives can be formed into topical dosage forms such as creams, ointments, sprays, and the like. Bercedin can also be formed into solid oral or non-oral dosage units such as, for example, tablets, capsules, powders, and suppositories, and may additionally include excipients, including without limitation lubricant(s), plasticizer(s), colorant(s), absorption enhancer(s), bactericide(s), and the like. Modes of administration include topical, oral and enteral, intravenous, intramuscular, subcutaneous, transmucosal (including rectal and buccal), and by-inhalation routes. Where applicable, such as, for

example, to treat skin or ocular lesions or localized ailments of various types, a topical route is preferred. When indicated, an oral or transdermal route is used (i.e., via solid or liquid oral formulations, or skin patches, respectively). For treatment of systemic infections such as, e.g., HIV, oral or intravenous administration may be used.

The amount of the agent to be administered may also depend upon the mode of administration. For topical administration, formulations containing Bercedin, preferably 100% Bercedin, if desired in granular or powder form, may be applied to an affected area between one and three times a day. It is preferable to use 100% Bercedin in the topical application, as inclusion of water, diluents and excipients may decrease its effectiveness. A layer of Bercedin is spread over the affected area. Treatment can be continued as long as the condition persists. For systemic administration, formulations may contain between about 10% and about 100% (w/v) Bercedin. The dosages for oral, mucosal, or intravenous systemic administration may range from about 20 to about 40 g of active ingredient (i.e. Bercedin) per patient per day, preferably about 30 g/patient/day. Topical and systemic administration can be used conjointly in which case the systemically delivered dosage can be decreased, if desired.

Treatment can last until the condition to be treated is abated or eliminated or the symptoms subside (it is recommended that for most pathologies treatment continue for 45-60 days) but may be continued for a longer time, even indefinitely due to the lack of toxicity and of the substance and its total clearance from the body. It will be understood that the pharmaceutical formulations of the present invention need not in themselves contain the entire amount of the agent that is effective in treating the disorder, as such effective amounts can be reached by administration of a plurality of doses of such pharmaceutical formulations.

In another embodiment, a method for treating infection comprises administering, in addition to Bercedin, other conventional bioactive substances that may act additively or synergistically with Bercedin to achieve a therapeutic effect. This may be achieved by administering a single formulation that comprises Bercedin and other bioactive substances, or by administering different formulations during the same time period. In the case of, for example, antibacterial therapy, a combination therapy would provide a clinical advantage in reducing the overall administration of antibiotics (thus lessening the development of antibiotic-resistant strains). Antibiotics that can be used in conjunction with Bercedin in the methods and compositions of the present invention

include without limitation penicillins (such as ampicillin, amoxicillin, methicillin, and the like), cephalosporins, aminoglycosides (such as streptomycin, neomycin, kanamycin, gentamicin, and the like), tetracyclines, chloramphenicol, and vancomycin. Conjoint Brcedin and antibiotic therapy is also preferred for HIV infection.

In one embodiment, a method for treating HIV infection comprises a 60-day treatment regimen in which the following are administered in combination (though not necessarily simultaneously as will be explained below): Oral Bercedin (30 g/day); Vitamin C (1 g/day) preferably intravenously; Streptomycin sulfate (3 g/day, 1 g being administered preferably orally every 8 hours); and a multi-vitamin supplement including trace elements, such as Supradyn (Roche) 1/day. It is believed that this treatment specifically acts on intestinal HIV target cells that are thought to act as reservoirs for the virus, and thereby reduce viral burden. The vitamin C is preferably administered substantially simultaneously with Bercedin (e.g., within one hour before or after administration of Bercedin) as such administration augments the effectiveness of Bercedin. The antibiotic (which may be replaced by another broad spectrum antibiotic) is used to prevent opportunistic infection, and the multivitamin supplement is to guard against vitamin deficiency. In general, the active ingredients other than Bercedin are not necessary but this regimen represents a preferred mode of treating HIV. Treatment with this regimen results in decrease of severity or elimination of symptoms directly attributable to HIV infection, decrease in severity or disappearance of opportunistic infections and secondary symptoms and in one confirmed case reverse seroconversion occurred.

For treating other viral infections, such as herpes genitalis, or herpes simplex, treatment commences when symptoms appear, and is continued at least until they are eliminated (or for 45-60 days). Topical application of Bercedin can be combined with systemic administration each following the regimen described above. Typically, treatment with Bercedin results in decrease of the duration and severity of the flare up and, often, avoidance of recurrence.

For treating hepatitis A or B systemic administration of 30g/day (orally) for two months has consistently resulted in elimination of the hepatitis A and in avoidance of relapses in the case of hepatitis B for prolonged periods of time (a total of 8 patients have been treated for hepatitis B, and none have experienced recurrence: two have been followed for 12 years, one for 11 years, and 3 for six years).

For treating influenza, Bercedin may be administered orally preventively during the influenza season, or therapeutically upon appearance of symptoms (treatment may be stopped after 3 days but is preferably continued for 7 days or longer). Scores of patients treated therapeutically report an immediate decrease in the duration and severity of influenza symptoms (aches, congestion, fatigue, fever) with the condition lasting only 3-4 days.

The foregoing demonstrate that Bercedin has remarkable antiviral (including antiretroviral) properties.

Additionally, topical application of the compositions of the present invention to unhealed wounds of varying causes consistently resulted in:

a) Prevention and/or reduction in bacterial contamination of the wound;

b) Rapid granulation;

c) Increased vascularization of the wound;

d) Expulsion or centralization of the necrotic threads or threads that are in an early stage of necrosis;

e) An increase in the protein level from 100 μg to 500 μg within 24 hrs after initial application; and

f) An increase in the hemoglobin level from 50 μg to 150 μg.

Histologically, there is an increase of collagen fibers and the inflamed infiltrate expands, gradually diffusing through the deep dermis, while the surrounding epidermis presents discrete papillae and acanthosis. The wounds were caused by perforated appendix, plantar perforation (secondary to diabetes) decubitus ulcers, abdominal penetrating wounds, metatarsal diabetic ulcers, dura mater infection, scrotal infection, and rectum-perineal fistula.

In dermic lesions caused by Hansen's disease, application of the compositions of the present invention results in proliferation of the microvasculature, reactivation of interstitial elements such as fibroblasts, reticulocytes, and collagen, and increase in granulation threads. There is also epithelial proliferation through the depth of the lesion to form a basal epithelial layer. Feeling and sensitivity to pain and pressure return to the affected area. In all cases the wounds had persisted for less than 5 years.

The foregoing demonstrate significant efficacy of Bercedin in wound healing including healing of infected wounds for which conventional therapies are either nonexistent or had previously failed.

Analysis and Identification of Active Principles

The active principle or principles in Bercedin that are responsible for the different therapeutic effects of the composition are not yet known. For example, the extent to which bioactivity of the composition resides in specific saccharide or polysaccharide moieties, and the possible contribution of amino acids or proteinaceous material, is still unclear.

The present invention encompasses the identification of active ingredients and/or combinations of ingredients in Bercedin, as well as the elucidation of critical proportions of one or more ingredients. This type of analysis enables the optimization of different formulations comprising Bercedin and Bercedin derivatives for treating different pathological conditions.

To achieve this end, Bercedin is subjected to fractionation and purification using methods well-known in the art of natural product biochemistry, including without limitation: crystallization; distillation (including fractional and steam distillation); filtration; centrifugation; differential extraction (using organic and aqueous solutions, including acids and bases); and chromatography, including molecular sizing, partition, countercurrent distribution, high-pressure liquid (using reverse-phase and silica-based resins), ion-exchange, and adsorption chromatography (using lipophilic or hydrophobic phases, or solid-phase lectins).

Typically, fractions are collected and analyzed for (i) their physical-chemical nature and (ii) their bioactivity. To identify the compounds present in each fraction, spectroscopic methods may be used, such as, for example, mass spectrometry (MS) (including high-resolution electrospray MS, laser desorption methods, time-of-flight methods, and fast-action bombardment using a variety of matrices); high-resolution nuclear magnetic resonance spectrometry (including proton, carbon, and nitrogen modes); infrared; ultraviolet; and visible spectrometry.

The above methods may be used in conjunction with qualitative analytical methods, such as, in the case of complex oligosaccharides, partial degradation with acids or bases, and/or digestion with degradative enzymes of defined substrate specificities.

Bioactivity of different fractions can be monitored by *in vivo* or *in vitro* methods. For example, culture systems well-known in the art can be used to quantify bacterial or viral infectivity. Typically, a stock culture of a particular bacterium or virus is mixed with the fraction being tested, after which the mixture is contacted with a suitable

host cell, and infection is monitored by cytopathology or lysis of the host cell. Similarly, *in vivo* animal model systems can be used to monitor tumor cell invasiveness. Typically, a defined amount of tumor cells are contacted with the fraction being tested, after which the mixture is introduced into an animal (into, e.g., a subcutaneous or peritoneal site) and the appearance of metastatic tumors is monitored. Other model systems are well-known in the art for monitoring, e.g., wound healing, effect of toxins, and the like.

It will be understood that the information obtained from fractionation, biochemical analysis, and bioassays can be combined in order to formulate Bercedin derivatives for different therapeutic purposes. For example, a particular oligosaccharide component may be more active (on a weight percentage basis) than unfractionated Bercedin for promoting wound healing; accordingly, this derivative would be formulated at an appropriate concentration into a topical cream or spray for clinical use.

The methods, tables and examples provided below are intended to describe preferred embodiments of the invention more fully and to demonstrate its advantages and applicability without limiting its scope.

Example 1: Confirmation of Antibacterial Activity of Bercedin in Vitro

The following experiments were performed to test the antibacterial action of Bercedin.

Petri dishes containing nutrient agar were seeded with either *E. coli* or *S. aureus* cultures. Increasing doses of Bercedin were applied to the dishes: 50, 100, 150, 200, 250, 300, 350, and 1000 mg. To control diffusion of Bercedin from the point of application, doses were also applied on filter-paper disks (13 mm diameter), or within Oxford glass (1 cm high, 1mm thick, and 6 mm wide). For *S. aureus*, additional samples were applied in an anaerobic environment. Following application of Bercedin, the plates were incubated for increasing times at 37°C.

The results indicated that doses of Bercedin of 200 mg and above were able to significantly inhibit the growth of both *E. coli* and *S. aureus* (the latter under both aerobic and anaerobic conditions) immediately after its introduction into the Petri dish. The entire microorganism population was killed within 24 hours.

Example 2: Use of Bercedin to Treat HIV Infection

In this study, 15 HIV-positive patients were treated according to the methods of the present invention.

I. Patient Group

Classification of the patient group was according to the Centers for Disease Control classification. 12 of the patients were male and 3 were female. The age distribution was as follows: 20-25, 5; 26-30, 4; 31-35, 3; 36-40, 1; and 41-45, 2. The distribution of known risk factors for HIV infection were as follows: 6 were bisexual, 3 were homosexual, and one was an intravenous drug user.

Diagnosis:

The diagnosis and staging of HIV infection followed the CDC system. 9 patients were classified in Group IV, subgroup C, category C1 (infectious secondary diseases); 2 were in Group IV, subgroup A (general illness); and 4 were in Group II (asymptomatic infection).

Symptoms:

Of the 9 patients in Group IV, subgroup C, all had lost more than 10% of their body weight within two to three months of the manifestation of the illness. 7 patients had diarrhea lasting more than 1 month. 6 patients had fever between 38-39°C for more than 1 month. All had occipital and axial lymph nodes that were more than 1 cm in diameter, hard, mobile and not painful. 6 patients had oral pharyngeal *Candida* infection (i.e. thrush). 2 patients had Herpes Zoster, one frontal and one on the thorax. 4 patients showed irritability. 2 patients had frontal-occipital head pain. 1 patient had asthenia.

Both of the patients in Group IV, subgroup A, exhibited the following symptoms: occipital and axial cervical adenitis of more than 1 cm and for more than one month; sporadic diarrhea; and night sweats.

The 4 patients in Group II exhibited positive micro-ELISA tests and positive Western blots.

Laboratory Data:

All of the patients had two tests using micro-ELISA which were strongly positive, and had confirmatory positive Western Blots, including GAG and ENV bands.

II. Treatment method:

The patients were administered the regimen described above as preferred for treating HIV infection for two months.

III. Results

A. Symptoms:

1. Patients in Group IV, subgroup C (AIDS)

Regression of the primary symptoms: Diarrhea was inhibited after three or four days from the start of the treatment without the use of any anti-cholinergics; the patients then recovered from dehydration due to the diarrhea by normal consumption of liquids. Fever also disappeared after three or four days in the absence of anti-fever medication. Body weight increased by 15 to 35 pounds (in some cases more) within a time period of two months.

Regression of secondary symptoms: Oral Yeast infection diminished after 8 days of treatment with anti-fungal agents. Infection and pain caused by Herpes zoster diminished after 15 days of treatment according to the invention. Cervical, occipital, and axial adenitis regressed by 0.6 mm after 15 days of treatment; the axial lesions rapidly returned to a normal stage, while the remaining lesions required approximately 45 days from the start of treatment.

2. Patients in Group IV, subgroup A (AIDS Complex)

Diarrhea disappeared after 3-4 days of treatment without using any anti-cholinergics. Night sweats also disappeared after 3 or 4 days. Lymph nodes swelling disappeared by the end of the treatment period. There is a slight increase in body weight (5 pounds on average).

3. Patients in Group II (Asymptomatic)

We observed a slight increase in body weight; good physical and mental state; and lymph nodes in a normal state in these patients.

B. Laboratory Findings

1. Blood Chemistry

Hemoglobulin counts, which were low at the beginning of the treatment, increased up to 13 or 14 gm/mm3 in most of the patients. Platlets, which were 60,00 to 70,000/mm3, became normal within 8 days of starting the treatment, eliminating the

gingival bleed exhibited by the patients. Leucopenia was improved substantially. TSGP and TSGO were normal.

In some patients, there was a slight increase in cholesterol, and the others remained normal. Triglycerides had a slight tendency to increase to the maximal normal limit.

2. Specific Tests

ELISA results continued to be weakly positive. Western blots also remained weakly positive, but the P24 band (which has some predictive value for the control of the infection) was not found.

IV. Conclusions:

The use of the methods of the present invention to treat HIV infected patients had the following outcomes:

1. A regression of symptoms was observed in all symptomatic patients. Primary and secondary symptoms disappeared. Body weight increased, and when the treatment was completed the patients had an excellent physical and mental state.

Two of the patients had surgery during the study. The first one had a diagnosis of acute abdominal infection caused by a perforated appendix. The second one had a left side inguinal hernia. Both cases were resolved satisfactorily.

2. The laboratory data showed that the blood chemistry became normal, the low platelet count was improved and there was an increase in triglycerides.

3 . ELISA and Western blot tests remained positive. The absence of P24 indicated reduction in the intensity of HIV infection.

4. Long term prognosis: Of the 9 patients with AIDS, 1 patient has survived four years longer than expected and remains asymptomatic with seroconversion; 2 patients have survived two years also remaining asymptomatic; and 6 patients have survived one year without symptoms. All of the four asymptomatic patients have remained mentally and physically normal. Of the two patients with AIDS related complex have remained asymptomatic.

In summary, this study supports the use of the methods of the present invention to treat HIV infection.

Example 3: Reverse seroconversion in an HIV-infected individual subsequent to Bercedin treatment

JAM, a 35-year old female, was admitted for an ectopic pregnancy. During surgery, she was given three units of blood. After this time, she showed the following symptoms: diarrhea, insomnia, fatigue, night sweats, weight loss of 3 kg body weight and nervous perturbation.

A micro-ELISA gave a highly positive result. The sample was sent to the Atlanta CDC, and the following report was obtained: HTL 3 Antibody (by Abbott) 0.863 positive strong and positive Western Blot (+.+.). When the sample was taken the patient was considered clinically asymptomatic.

Due to the positive indication of infection she was treated according to the present invention for 8 months, using the regimen described above for 60 days.

The development of her Micro-ELISA results was as follows:

SAMPLE TAKEN	RESULTS
Time 0	highly positive
3 months	medium positive
7 months	weak positive
15 months	negative
23 months	negative
25 months	negative

The patient has remained asymptomatic since the time of treatment (nine years). The reversal of the micro-ELISA titer strongly suggests that the treatment according to the invention dramatically reduced any HIV infection.

Example 4: Clinical study to evaluate efficacy of Bercedin in treating HIV infection

The following study is performed to evaluate the clinical effects of Bercedin treatment on individuals suffering from HIV infection.

Prior to enrolling in the study, HIV-negative and HIV-positive patients receive a general physical examination and blood will be drawn for a CBC, ESR, lymphocyte panel, bDNA viral load, and chemistry screen with lipids. A routine urinalysis will also be performed. Inclusion criteria include: more than 18 years of age; for normal subjects, HIV negative and in apparent good health; for HIV-positive subjects,

having a detectable viral load by bDNA evaluation and having CD4+ lymphocyte counts below 500 cells/mm^3. Exclusion criteria include: use of AZT or other anti-retroviral medications, including protease inhibitors; use of prophylactic antibiotics other than Trimethoprim/sulfanemethoxazole; hepatitis or known significant liver abnormality (detected by physical exam, history, or serum GGt, ALT, and AST enzyme levels); renal abnormalities (detected by physical exam, history, serum BUN and creatinine levels, or urinalysis); pregnancy or breast-feeding; diabetes or volatile blood glucose and triglycerides; and pancreatitis. In addition to CBC and comprehensive blood chemistries, subjects will be evaluated with respect to CD4+ T-cells; CD8+ T-cells; NK cells; CD4/DC8 ratio; and B cells.

Subjects ingest 30 g/day of Bercedin (in 1-g gelatin capsules). On days 8, 22, 43, and 64, subjects provide urine for a routine urinalysis and approximately 20 ml of blood is collected for CBC and chemscreen. On days 43 and 64, additional blood is collected for lymphocyte panel and viral load analysis.

The primary outcome variables are:

Frequence and severity of adverse events, including:

- new signs or symptoms
- significant increases in severity of present signs or symptoms
- significant changes in lab tests from baseline

Changes in viral load (branched chain DNA) in HIV-positive subjects

Changes in lymphocyte populations

- Total
- CD4+ (total and relative)
- CD8+ (total and relative)
- CD4+/CD8+ ratio
- NK cells
- B cells (total and relative)

Secondary outcome variables include other laboratory changes, as well as weight changes.

CLAIMS

1. A pharmaceutical formulation comprising Bercedin and optionally a pharmaceutically acceptable carrier or diluent.

2. A formulation as defined in claim 1, wherein said Bercedin comprises from about 10 to about 100 percent by weight of the total formulation.

3. A dosage form comprising a formulation as defined in claim 1, wherein said dosage form is selected from the group consisting of creams, ointments, sprays, tablets, capsules, powders, and suppositories.

4. A method for treating a pathological condition in a mammal in need of such treatment, said method comprising administering to said mammal Bercedin in an effective amount and for a time sufficient to treat said condition.

5. The method of claim 4, wherein said administering is achieved using a route selected from the group consisting of oral, enteral, intravenous, intramuscular, subcutaneous, transmucosal, transdermal, and by-inhalation routes.

6. The method of claim 4, wherein said condition is caused by inflammation, bacterial or viral infection, or a toxin.

7. The method of claim 4, wherein said condition is selected from the group consisting of dermic lesions produced by Hansen's disease, diabetic foot ulcers, diabetic gangrene, perineal necrotizing cellulitis, intestinal fistula, rectum-perineal fistula, eviscerations, decubital and vascular source ulcers, acne, and infection with herpes simplex, herpes genitalis, influenza and cold viruses, Hepatitis A and B, and human immunodeficiency virus (HIV).

8. A method for producing Bercedin, which comprises the steps of:

(i) macerating sugar cane to produce a liquid extract, and filtering the extract;

(ii) heating the filtered liquid extract obtained in (i) at a temperature of from about 60°C to about 70°C for a time period of about 30 to about 60 minutes;

(iii) heating the filtered liquid extract obtained in (ii) at a temperature of from about 130°C to about 165°C for about 24 hours, wherein said extract is subjected to constant and uniform shaking during the heating; and

(iv) cooling the extract obtained in (iii) to obtain a solid.

9. An extract from sugar cane, which is produced by:

(i) macerating sugar cane to produce a liquid extract, and filtering the extract;

(ii) heating the filtered liquid extract obtained in (i) at a temperature of from about 60°C to about 70°C for a time period of about 30 to about 60 minutes;

(iii) heating the filtered liquid extract obtained in (ii) at a temperature of from about 130°C to about 165°C for about 24 hours, wherein said extract is subjected to constant and uniform shaking during the heating; and

(iv) cooling the extract obtained in (iii) to obtain a solid.

10. A pharmaceutical composition comprising

(i) an extract as defined in claim 9 and

(ii) a pharmaceutically acceptable carrier or diluent.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/13427

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) : A01N 43/04; A61K 31/715
US CL : 514/23, 53, 54
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/23, 53, 54

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,055,455 A (PIER, G.B.) 08 October 1991, see entire document, especially from column 3, line 29 to column 4, line 4.	1-10
A, E	US 5,547,674 A (KHWAJA, T.A.) 20 August 1996, see entire document.	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
30 SEPTEMBER 1997

Date of mailing of the international search report
30 OCT 1997

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Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/13427

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CA, CAPLUS, BIOSIS, MEDLINE, EMBASE, WPIDS, USPATFULL search terms: bercedin, (sugar cane and (bacteria? or viral or fungal or toxin or trauma or burn)), hasen or diabet? or cellulitis or fistula or ulcer or herpes or hepatitis or influenza or hiv or human immunodeficienc? or acne), inflammation# or anti-inflammator####, ####saccharide or ####saccharides or oligosaccharid###, sugar(5a)extract####, lectin#, amino acid#